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Multisite Skin Biopsies vs Cerebrospinal Fluid for Prion Seeding Activity in the Diagnosis of Prion Diseases

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IMPORTANCE Recent studies have revealed that autopsy skin samples from cadavers with prion diseases (PRDs) exhibited a positive prion seeding activity similar to cerebrospinal fluid (CSF). It is worthwhile to validate the findings with a large number of biopsy skin samples and compare the clinical value of prion seeding activity between skin biopsies and concurrent CSF specimens.

OBJECTIVE To compare the prion seeding activity of skin biopsies and CSF samples and to determine the effectiveness of combination of the skin biopsies from multiple sites and numerous dilutions on the diagnosis for various types of PRDs.

DESIGN, SETTING, AND PARTICIPANTS In the exploratory cohort, patients were enrolled from September 15, 2021, to December 15, 2023, and were followed up every 3 months until April 2024. The confirmatory cohort enrolled patients from December 16, 2023, to June 31, 2024. The exploratory cohort was conducted at a single center, the neurology department at Xuanwu Hospital. The confirmatory cohort was a multicenter study involving 4 hospitals in China. Participants included those diagnosed with probable sporadic Creutzfeldt-Jakob disease or genetically confirmed PRDs. Patients with uncertain diagnoses or those lost to follow-up were excluded. All patients with PRDs underwent skin sampling at 3 sites (the near-ear area, upper arm, lower back, and inner thigh), and a portion of them had CSF samples taken simultaneously. In the confirmatory cohort, a single skin biopsy site and CSF samples were simultaneously collected from a portion of patients with PRDs.

EXPOSURES The skin and CSF prion seeding activity was assessed using the real-time quaking-induced conversion (RT-QUIC) assay, with rHaPrP90-231, a Syrian hamster recombinant prion protein, as the substrate. In the exploratory cohort, skin samples were tested at dilutions of 10^{-2} through 10^{-4}. In the confirmatory cohort, skin samples were tested at a dilution of 10^{-2}. A total of four 15-µL wells of CSF were used in the RT-QUIC assay.

MAIN OUTCOMES AND MEASURES Correlations between RT-QUIC results from the skin and CSF and the final diagnosis of enrolled patients.

RESULTS In the exploratory cohort, the study included 101 patients (mean [SD] age, 60.9 [10.2] years; 63 female [62.4%]) with PRD and 23 patients (mean [SD] age, 63.4 [9.1] years; 13 female [56.5%]) without PRD. A total of 94 patients had CSF samples taken simultaneously with the skin biopsy samples. In the confirmatory cohort, a single skin biopsy site and CSF sample were taken simultaneously in 43 patients with PRDs. Using an experimental condition of 10^{-2} dilution, the RT-QUIC positive rates of skin samples from different sites were comparable with those of the CSF (skin: 18 of 26 [69.2%] to 74 of 93 [79.6%] vs CSF: 71 of 94 [75.5%]). When tested at 3 different dilutions, all skin sample positivity rates increased to over 80.0% (79 of 93 for the near-ear area, 21 of 26 for the upper arm, 77 of 92 for the lower back, and 78 of 92 for the inner thigh). Combining samples from skin sites near the ear, inner thigh, and lower back in pairs yielded positivity rates exceeding 92.1% (93 of 101), significantly higher than CSF alone (71 of 94 [75.5%]; P =.002). When all skin sample sites were combined and tested at 3 dilution concentrations for RT-QUIC, the sensitivity reached 95.0% (96 of 101). In the confirmatory cohort, the RT-QUIC positive rate of a single skin biopsy sample was slightly higher than that of the CSF (34 of 43 [79.1%] vs 31 of 43 [72.1%]; P = .45).

CONCLUSIONS AND RELEVANCE Results of this diagnostic study suggest that the sensitivity of an RT-QUIC analysis of a combination of 2 or more skin sites was superior to that of CSF in diagnosing PRDs.

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Supplemental content

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Human prion diseases (PRDs) are transmissible, progressive, and invariably fatal neurodegenerative disorders characterized by the accumulation of misfolded prion protein (PRPSC) in the central nervous system through the seeding and conformational conversion of normal prion protein. These diseases include Creutzfeldt-Jakob disease (CJD), fatal familial insomnia (FFI), Gerstmann-Sträussler-Scheinker syndrome, kuru, and variably protease-sensitive prionopathy. Sporadic CJD (SCJD) is the most common human PRD. The definite diagnosis of SCJD still depends neuropathological examination of PRPSC in the brain currently, which is inconvenient for the diagnosis clinically.

The development of real-time quaking-induced conversion (RT-QUIC) technology marks a revolutionary shift in the diagnostic approach to PRDs. RT-QUIC boasts an impressive 99% to 100% specificity, providing diagnostic accuracy comparable with traditional neuropathological methods.[1-3] Cerebrospinal fluid (CSF) is the most common specimen for RT-QUIC; however, there are some clinical situations that may influence CSF availability, such as invasiveness, contraindications, and patient refusal. In 2017, a groundbreaking study[4] successfully applied skin RT-QUIC for CJD diagnosis. Subsequent studies[5,6] have showed that the sensitivity of skin RT-QUIC is comparable with, or even higher than, that of CSF. In prion-infected rodent models, detection of skin PRPSC-seeding activity (SA) precedes the evident brain neuropathology and clinical symptoms by a significant margin.[7] Skin PRPSC in prion-infected animals is also coincidental with the implementing antiprion compounds, suggesting a possible biomarker for prion therapeutics.[8] A more recent study[9] using large-size skin samples from cadavers has verified the promising clinical significance of skin RT-QUIC in the diagnosis of human PRDs. For clinical practice, it is crucial to evaluate the diagnostic values and optimize the detection scheme of the skin RT-QUIC using large-size skin biopsy samples.

In this study, we prospectively collected the biopsy sample size for PRDs and compared the diagnostic efficiency of CSF and multisite skin in vivo to explore the potential of replacing CSF with biopsied skin samples as the preferred specimen for RT-QUIC detection. In addition, the possible association of skin or CSF RT-QUIC positivity with other demographic, clinical, and laboratory characteristics was accessed.

Key Points

Question Are misfolded prion protein aggregates in skin biopsies a more sensitive diagnostic biomarker for prion diseases (PRDs) compared with those in the cerebrospinal fluid (CSF)?

Findings In this diagnostic study involving 415 skin samples and 160 CSF samples from 101 patients with PRDs and 23 patients without PRDs, the sensitivity of single-site skin biopsies was comparable with that of the CSF. However, the combination of 2 or 3 skin biopsies exhibited greater diagnostic sensitivity compared with the CSF alone.

Meaning Results suggest that analysis of 2 or more skin sites was superior to CSF analysis for diagnosing PRDs and may be valuable for patients with negative CSF real-time quaking-induced conversion assay results or those unable or unwilling to undergo lumbar puncture.

Methods

Study Design

This study was monitored and approved by the institutional review boards (IRBs) of the Xuanwu Hospital, Capital Medical University, Beijing, China. The use of human tissues was authorized by the IRB. Written informed consent was obtained from all living participants undergoing skin biopsy or their guardians. The study was carried out in compliance with the principles of the Declaration of Helsinki. This study followed the Standards for Reporting of Diagnostic Accuracy (STARD) reporting guidelines.

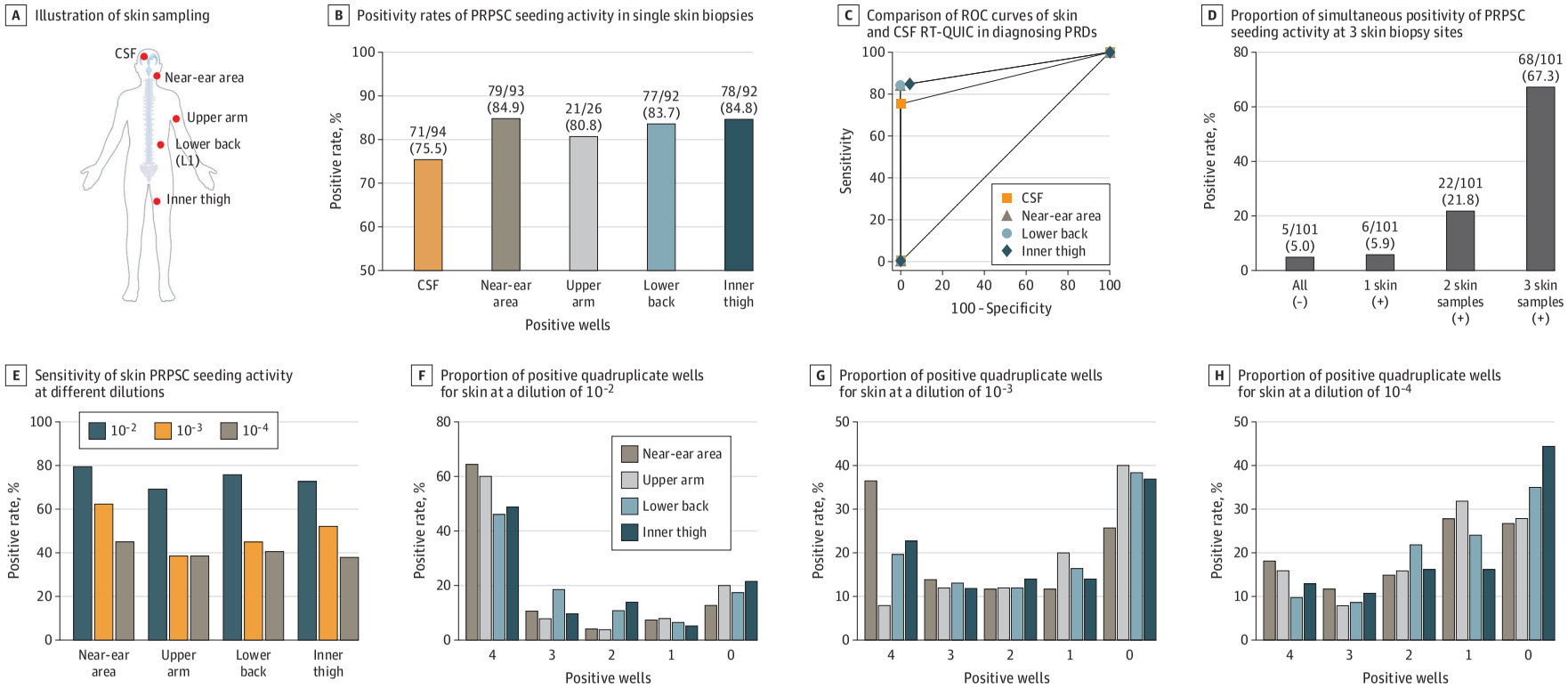
In the exploratory cohort, patients suspected as having PRDs who were admitted to the neurology department at Xuanwu Hospital from September 15, 2021, to December 15, 2023, were prospectively enrolled. All participants were Han Chinese. This information was identified based on the patients' ethnicity as recorded on their national identification cards. Han Chinese is the largest ethnic group in China. The diagnosis of SCJD was mainly based on the current World Health Organization criteria[10] and referred with the proposed diagnostic criteria for MM2-cortical type[11] or MM2-thalamic type.[12] MM2 refers to methionine homozygous (MM) at codon 129 and is associated with a specific type of pathologic prion protein, PRPSC type 2. All cases of genetic PRD were verified by *PRNP* sequencing to contain a definite gene variant on 1 allele. Patients were regularly followed up every 3 months to ensure precise diagnosis and monitor changes in their conditions, with the most recent follow-up until April 2024. Patients with alternative diagnoses or those lost to follow-up were excluded. Additional patients with other neurological disorders meeting clinical diagnostic criteria were enrolled as the control group without PRDs.[13-17]

In the confirmatory cohort, patients with PRDs, including probable SCJD and genetic PRD, were prospectively enrolled from 4 hospitals in China between December 16, 2023, and June 31, 2024.

Skin Biopsy

After disinfection with povidone-iodine, patients received local anesthesia via subcutaneous injection of 1% to 2% lidocaine hydrochloride. Disposable 3-mm punches (Acu Punch [Acuderm Inc]) were used to collect skin biopsies from the following areas: the near-ear area (approximately 1 cm away from the ear), upper arm (10 cm above the elbow), lower back (5 cm from the midline at the first lumbar vertebra), and inner thigh (20 cm above the medial patella) (**Figure 1**A). These biopsies included the epidermis, dermis, and adipose tissues. In the exploratory cohort, a random number generator was used to select 3 of 4 skin sites in the first 35 patients with PRDs. The upper arm had the lowest positivity rate; therefore, in subsequent patients, samples were taken from the near-ear area, lower back, and inner thigh.

Figure 1. Diagnostic Performance of Skin and Cerebrospinal Fluid (CSF) Misfolded Prion Protein Seeding Activity (PRPSC-SA) in Prion Diseases (PRDs)



A, Illustration of skin sampling. B, Positivity rates of PRPSC-SA in single skin biopsies, considered positive if positive at any dilution from 10^{-2} to 10^{-4}, compared with CSF in PRDs. Although the positivity rates for the area near the ear and the lower back were higher than those for the CSF, the differences did not reach statistical significance. C, Comparison of receiver operating characteristic (ROC) curves of skin and CSF real-time quaking-induced conversion (RT-QUIC) assay in diagnosing PRDs. D, The diagnostic efficacy of the near-ear area and lower back tended to be higher than CSF (P = .08-.09). E, Sensitivity of skin PRPSC-SA at different dilutions. The near-ear area exhibited the highest sensitivity to PRPSC-SA at any dilution from 10^{-2} to 10^{-4}. F-H, Proportion of positive quadruplicate wells for skin at dilutions 10^{-2} (F), 10^{-3} (G), and 10^{-4} (H), with the near-ear area showing the highest ratio of positive wells.

The main risks associated with skin biopsies include bleeding, local infection, and allergic reactions to local anesthetics, among others. In our study, there were 2 patients who experienced significant bleeding during the biopsy of the ear area due to the rich vascular distribution in that region. This was managed with continuous pressure application, which alleviated the bleeding without the need for sutures. Consequently, for all subsequent biopsies of the ear area, we applied pressure for 10 minutes after the procedure. After this adjustment, no further cases of significant bleeding were observed, only minor local oozing. No other risks, such as infection or allergic reactions, were encountered in our study.

Processing of Skin Tissues

Skin samples were washed 3 times with 1× tris-buffered saline, chopped, and prepared for 10% (weight per volume) skin homogenates in tris-buffered saline with 2mM $CaCl_2$ and 0.25% (weight per volume) collagenase A (Roche). After incubation at 37 °C for 4 hours with shaking, tissue homogenates were prepared with a homogenizer. The homogenates were centrifuged at 500*g* for 5 minutes, and the supernatants were collected for subsequent RT-QUIC assay.[7]

Preparation of Recombinant Prion Protein

The recombinant hamster PrP90-231 (rHaPrP90-231) protein was expressed and purified following established protocols[18] (eMethods in Supplement 1).

RT-QUIC Assays

All skin or CSF samples were blindly examined at the Chinese Center for Disease Control and Prevention. The RT-QUIC assay of PRPSC-SA of biopsied skin and CSF samples was conducted as previously described with minor modification.[18] In brief, the RT-QUIC reaction mixture consisted of 10 µg of rHaPrP90-231, 1 × PBS, 170mM NaCl, 1mM EDTA, 0.01mM thioflavin T, and 0.002% sodium dodecyl sulfate. Additionally, 15 µL of CSF samples or 2 µL of skin homogenates diluted from 10^{-2} to 10^{-4} were added into the mixtures, with a final volume of 100 µL. Each sample was analyzed in quadruplicate. The RT-QUIC reaction was carried out in a black 96-well plate with an optical bottom (Nunc, 265301) using a BMG FLUOstar plate reader (BMG Labtech). The standard operating conditions were as follows: temperature at 55 °C, vibration speed at 700 revolutions per minute, with a 60-second vibration followed by a 60-second incubation cycle, resulting in a total reaction time of 60 hours. Thioflavin T fluorescence, measured at excitation and emission wavelengths of 450 nm and 480 nm, respectively, was recorded automatically every 45 minutes and expressed as relative fluorescence units. Positive RT-QUIC reactivity of individual wells was defined as the mean fluorescence value of the negative controls plus 10 times the SD. A sample was considered positive when at least 2 wells showed positive reaction curves. The relative PRPSC-SA was extrapolated by plotting relative fluorescence unit readouts against assay time as follows: Tlag (the time interval between the beginning of the reaction and the time in which the curve of the fluorescent signal crosses the threshold), Fmax (the maximum thioflavin T fluorescence in the stationary phase), T50 (the time latency to obtain 50% of the maximum relative fluorescence), Vmax (the maximum slope of the amplification curve determined as the maximum increase in relative fluorescence over time), and RT-QUIC score[19,20] (eMethods in Supplement 1).

Statistical Analysis

This analysis was performed using SPSS, version 25.0 (IBM Corp). The Kolmogorov-Smirnov test was used to verify the normal distribution of continuous variables. The *t* test or 1-way analysis of variance followed by a post hoc Bonferroni test was used for comparison of normally distributed data. The Mann-Whitney *U* test or Kruskal-Wallis test was used to compare variables without a normal distribution. A combined result was considered as positive if any of the near-ear areas, upper arm, lower back, or inner thigh had a positive result. Categorical variables and paired categorical data were examined using the χ^2 test. The McNemar test was used to account for the paired nature of the samples. Continuous variables were reported as mean (SD) or median (IQR), and categorical variables were summarized using frequencies. MedCalc software was used for generating receiver operating characteristic (ROC) curves, calculating the area under the ROC curve (AUROC), and comparing the predictive performance of skin and CSF RT-QUIC using the method described by DeLong and colleagues.[21] Associations between RT-QUIC parameters and clinical parameters were calculated using partial Spearman correlation. For all analyses, significance was determined with a 2-sided P value < .05, which was Bonferroni-corrected for multiple comparisons.

Results

Demographic Characteristics of Individuals With and Without PRDs

In the exploratory cohort, the study included 101 patients (mean [SD] age, 60.9 [10.2] years; 63 female [62.4%]; 38 male [37.6%]) with PRD and 23 patients (mean [SD] age, 63.4 [9.1] years; 13 female [56.5%]; 10 male [43.5%]) without PRD. Ultimately, 86 patients were diagnosed with probable SCJD, whereas 15 individuals were identified as having genetic PRDs, comprising 13 cases of genetic CJD and 2 cases of FFI (as illustrated in eFigure 1 in Supplement 1). A total of 94 patients had CSF samples taken simultaneously with the skin biopsy samples. In the confirmatory cohort, a total of 43 patients (mean [SD] age, 61.7 [9.1] years; 26 female [60.5%]; 17 male [39.5%]) with PRDs, including 38 probable SCJD and 4 genetic PRDs (2 cases with E200K, 1 P102L, and 1 D178N-129M), were prospectively enrolled. Demographic and clinical data for the patients are summarized in **Table 1**.

In the exploratory cohort, 3 of 4 skin sites were selected randomly in the first 35 patients with PRDs. The upper arm had the lowest positivity rate; therefore, in the subsequent 66 patients, samples were taken from the near-ear area, lower back, and inner thigh. In total, 303 skin sites were sampled: 93 from the near-ear areas, 92 from the lower back, 92 from the inner thighs, and 26 from the upper arms. In the confirmatory cohort, all patients with PRDs underwent a single skin biopsy,

Table 1. Demographics, Clinical Features, and Diagnostic Tests of Patients

Characteristic	Exploratory cohort			Confirmatory cohort, PRDs (n = 43)
	PRDs (n = 101)	Non-PRDs (n = 23)	*P* value	
Patient information				
Age at onset, mean (SD), y	60.9 (10.2)	63.4 (9.1)	.28	61.7 (9.1)
Sex, No. (%)				
Female	63 (62.4)	13 (56.5)	.60	26 (60.5)
Male	38 (37.6)	10 (43.5)		17 (39.5)
Onset to RT-QUIC, median (IQR), d	93.0 (54.0-252.0)	570.0 (300.0-750.0)	<.001	138.5 (49.8-280.5)
Duration, median (IQR), mo	11.9 (3.5-18.6)	NA	NA	6.8 (4.7-10.9)
Clinical signs, No. (%)				
Cognitive	97 (96.0)	17 (73.9)	<.001	41 (95.3)
Psychiatric	55 (54.5)	11 (47.8)	.57	21 (48.8)
Visual	33 (32.7)	2 (8.7)	.02	12 (27.9)
Extrapyramidal	61 (60.4)	8 (34.8)	.03	25 (58.1)
Pyramidal	53 (52.4)	5 (21.7)	.008	24 (55.8)
Cerebellar	61 (60.4)	6 (26.1)	.003	27 (62.8)
Myoclonus	36 (35.6)	0	<.001	13 (30.2)
Mutism	2 (2.0)	0	>.99	2 (4.7)
CSF t-tau >1150 pg/mL, No. (%)	54/68 (79.4)	1/15 (6.7)	<.001	21/28 (75.0)
CSF 14-3-3 positive, No. (%)	66/94 (70.2)	2/10 (20.0)	.002	28 (65.1)
Periodic discharges on EEG, No. (%)	43 (42.6)	0/8	.02	18 (41.9)
Hyperintensity on DWI, No. (%)	94 (94.1)	0	<.001	41 (95.3)
Hypometabolism on PET, No. (%)	28/28 (100.0)	17/19 (89.5)	.16	20/21 (95.2)
Codon 129 MM genotype, No. (%)	99 (98.0)	NA	NA	43 (100.0)
Codon 129 MV genotype, No. (%)	2 (2.0)	NA	NA	0
Genetic prion diseases	15 (14.9)	NA	NA	4 (9.3)

Abbreviations: CSF, cerebrospinal fluid; DWI, diffusion-weighted imaging; EEG, electroencephalography; MM, methionine homozygous; MV, methionine/valine heterozygous; NA, not applicable; PET, positron emission tomography; PRD, prion disease; RT-QUIC, real-time quaking-induced conversion.

with 43 skin sites sampled: 30 from the near-ear areas, 6 from the inner thigh, 5 from the upper arm, and 2 from the back.

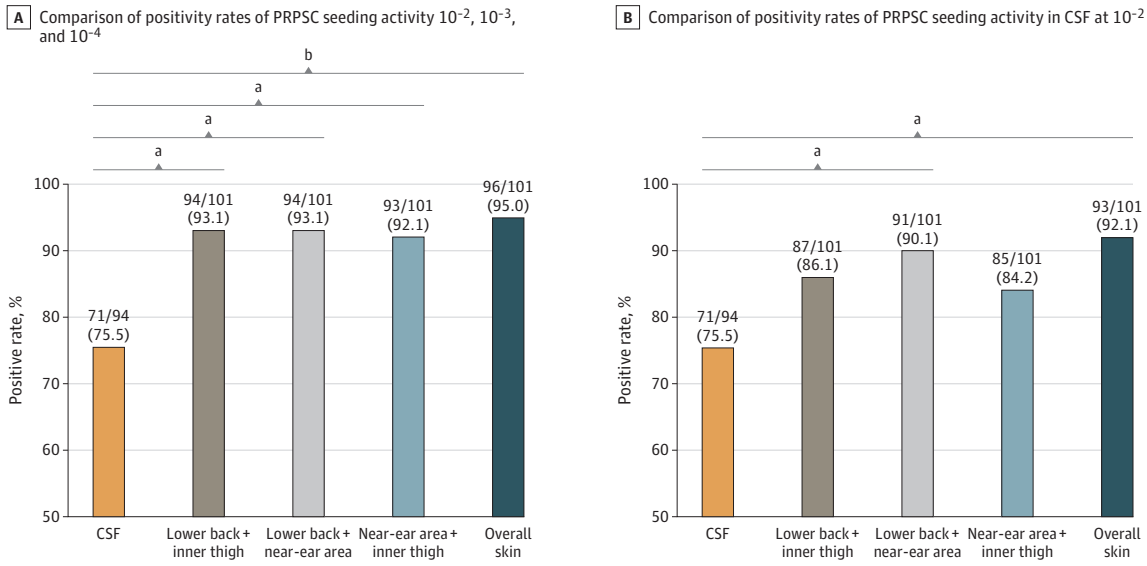
There were no significant differences in age and sex between the PRD and non-PRD groups in the exploratory cohort. However, significant differences were observed in the time from onset to biopsy, clinical symptoms, and auxiliary examination results between the 2 groups, as detailed in Table 1. During the study period, 58 patients (57.4%) with PRDs died, with a median (IQR) survival time of 11.9 (3.0-17.4) months. Additional information on the 23 patients without PRDs is provided in eTable 1 in Supplement 1.

Comparison of Diagnostic Efficacy Between Skin and CSF RT-QUIC

In patients with PRDs, the overall sensitivity of CSF RT-QUIC assays was 75.5% (71 of 94) in the exploratory cohort (Figure 1B). For SCJD and genetic PRDs, the sensitivities were 76.5% (62 of 81) and 69.2% (9 of 13), respectively. None of the patients without PRDs showed positive results in the CSF RT-QUIC assay, indicating a specificity of 100% for CSF RT-QUIC. In skin samples, the highest sensitivity of a single skin site was the near-ear area (84.9% [79 of 93]), followed by the inner thigh (84.8% [78 of 92]), lower back (83.7% [77 of 92]), and upper arm (80.8% [21 of 26]). The overall specificity of skin RT-QUIC was 95.7% (22 of 23), with specificities of 100% for the near-ear area and the lower back, and 95.7% (22 of 23) for the inner thigh (Figure 1B and C). The diagnostic efficacy of the near-ear area (AUROC, 0.93; 95% CI, 0.86-0.97) and the lower back (AUROC, 0.92; 95% CI, 0.85-0.96) tended to be higher than that of the CSF (AUROC, 0.88; 95% CI, 0.80-0.93), although the difference did not reach statistical significance (CSF vs near ear, $P = .09$; CSF vs lower back, $P = .08$) (eTable 2 in Supplement 1). Furthermore, 89.1% of patients (90 of 101) tested positive in at least two skin sites (Figure 1D). In the confirmatory cohort, the overall positive rate for individual skin biopsy samples was slightly higher than for the CSF (79.1% [34 of 43] vs 72.1% [31 of 43]) (eFigure 2 in Supplement 1), although this difference was not statistically significant. For SCJD and genetic PRDs, the sensitivities were 74.4% (29 of 39) and 50.0% (2 of 4) for the CSF and 79.5% (31 of 39) and 75.0% (3 of 4) for skin biopsy, respectively.

At a single skin site with a 10^{-2} dilution, the diagnostic sensitivity of skin was comparable with that of the CSF (skin: 18 of 26 [69.2%] to 74 of 93 [79.6%] vs CSF: 71 of 94 [75.5%]) (Figure 1E) in the exploratory cohort. However, considering 3 dilutions, the sensitivity improved by 5.4% (5 of 93) to 12.0% (11 of 92) across different skin sites. RT-QUIC tests on individual skin sites revealed a clear dose-dependent pattern, with positivity rates decreasing over an increase in sample dilution. The area near the ear exhibited the highest positivity rate and the highest proportion of 4 positive wells at any dilution concentration (Figure 1F-H). At a 10^{-2} dilu-

Figure 2. Evaluation of Multiple Skin Sites With the Real-Time Quaking-Induced Conversion (RT-QUIC) Assay and Diagnostic Sensitivity



Comparison of positivity rates of misfolded prion protein seeding activity (PRPSC-SA) in the cerebrospinal fluid (CSF), combination of 2 skin biopsy sites at dilutions of 10^{-2}, 10^{-3}, and 10^{-4} (A) and at dilutions of 10^{-2} (B). The sensitivity of diagnosing prion diseases (PRDs) from any 2 skin combined samples is superior to that of CSF alone ($P < .05$).

[a] $P < .01$.

[b] $P < .001$.

tion, all 4 sites demonstrated consistent results in quadruplicate PRPSC-SA tests.

Association of Number of Skin Sites With Diagnostic Efficacy of Skin RT-QUIC

We further evaluated the impact of combining multiple skin samples on the diagnosis of PRDs in the exploratory cohort. Our findings indicated that combining any 2 sites of the near-ear area, inner thigh, and lower back significantly improved the positivity rate compared with CSF alone (71 of 94 [75.5%]; P =.002) (**Figure 2**A). The highest sensitivity was 93.1% (94 of 101) for the lower back and inner thigh combination. The collective sensitivity of the skin samples from all sites was 95.0% (96 of 101), with 95.3% (82 of 86) in the group of SCJD and 93.3% (14 of 15) in that of genetic PRDs. Although combining 3 skin sites provided the highest diagnostic efficiency, it was not statistically superior to combining any 2 skin sites. At a 10^{-2} dilution, the highest positivity rate of 90.1% (91 of 101) was observed when combining skin samples from the near-ear area and lower back region (Figure 2B). Furthermore, combining CSF with any of the aforementioned skin sites was also superior to CSF alone (71 of 94 [75.5%]; P =.01) (eFigure 3 in Supplement 1). In the confirmatory cohort, the RT-QUIC positive rate of a single skin biopsy sample was slightly higher than that of the CSF (34 of 43 [79.1%] vs 31 of 43 [72.1%]; P = .45).

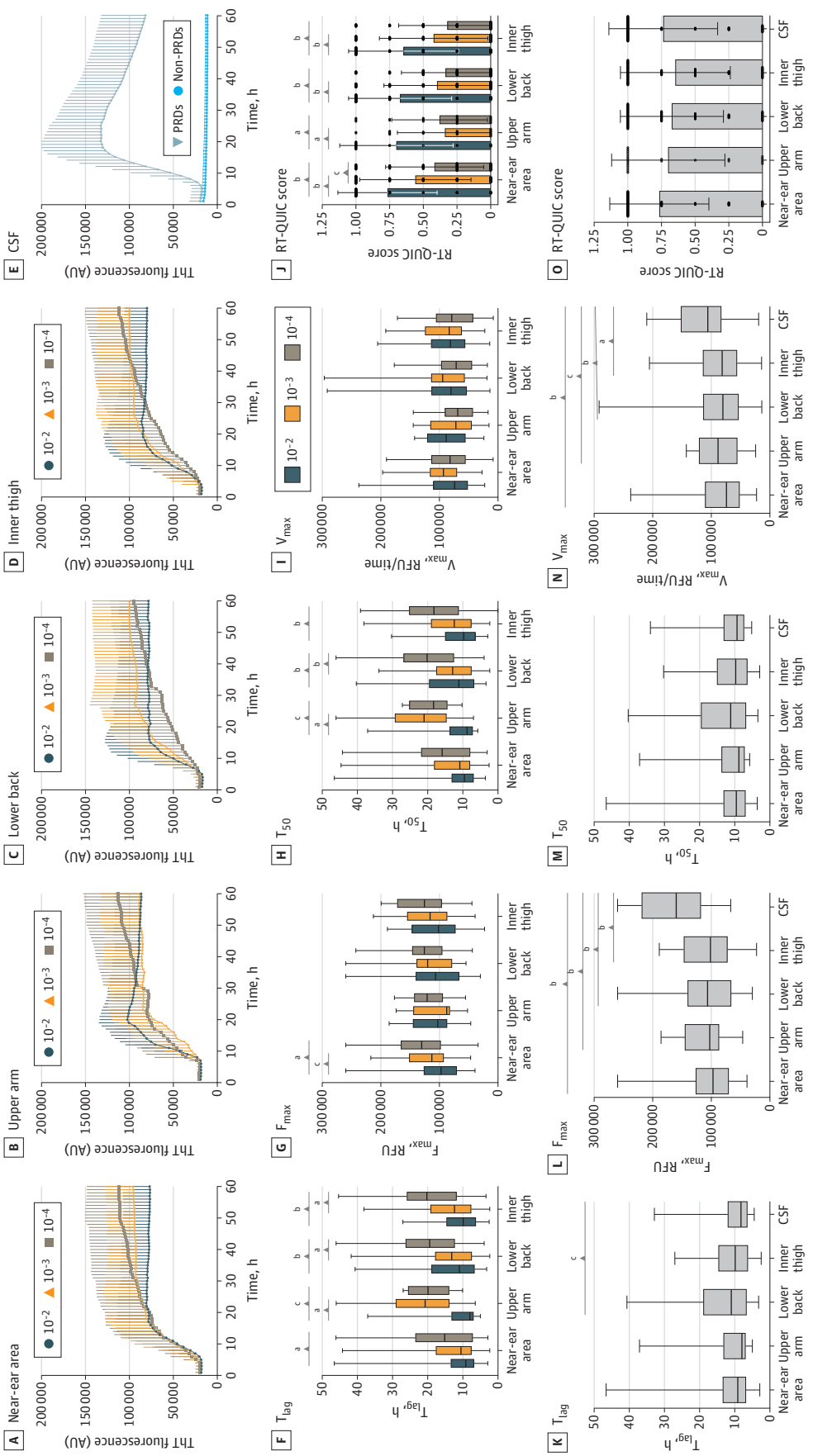
Association of Skin Homogenate Dilution With Different Skin Sites and CSF RT-QUIC

The RT-QUIC thioflavin T fluorescence kinetics of PRPSC over time for various skin sites and CSF in the exploratory cohort are illustrated in **Figure 3A-E**. Within the same skin site, response curves varied with different dilutions, showing an earlier Tlag (Figure 3F) and T50 (Figure 3H) at a 10^{-2} dilution along with higher RT-QUIC scores (Figure 3J). There was a general trend of increasing Fmax with higher dilutions across different sites, but this increase was only significant near the ear area (Figure 3G). Vmax at the end point of RT-QUIC reaction did not show significant differences across various skin tissues (Figure 3I). In CSF, both Fmax (Figure 3L) and Vmax (Figure 3N) were significantly higher compared with other skin sites. CSF showed an earlier Tlag compared with the lower back (Figure 3K), and there were no significant differences in T50 (Figure 3M) and RT-QUIC scores (Figure 3O) among other skin sites and the CSF. Additionally, no significant differences were observed in the amplification curves between different skin sites at the same dilution concentration (eFigure 4 in Supplement 1).

Correlation Between PRPSC-SA and Clinical Features

We analyzed the correlation between PRPSC-SA measured with skin samples from the near-ear area, lower back, and inner thigh at a dilution of 10^{-2}, along with CSF, and clinical characteristics in the exploratory cohort. After adjusting for age and sex, we observed a positive correlation between the RT-QUIC score of near ear area and CSF and the severity of cognitive impairment (Spearman ρ =0.430; P =.03 to Spearman ρ =0.496; P =.01) in patients (eTable 3 in Supplement 1). Additionally, Tlag and T50 measured on skin of the back and inner thigh exhibited a negative correlation between cognitive impairment (Spearman ρ =−0.504; P =.02 to Spearman ρ =−0.678; P <.001) and a positive correlation with Medical Research Council Prion Disease Rating Scale (Spearman ρ =0.425; P =.049 to Spearman ρ =0.458; P =.03) (eTable 4 in Supplement 1). However, none of the skin and CSF RT-QUIC parameters showed a significant correlation with prognosis.

Figure 3. Comparison of Real-Time Quaking-Induced Conversion (RT-QUIC) Thioflavin T (ThT) Fluorescence Kinetics of Misfolded Prion Protein (PRPSC) Over Time at Different Dilutions and Skin Sites



A-D, The RT-QUIC ThT fluorescence kinetics of PRPSC at different dilutions were compared for various skin sites: near-ear area (A), upper arm (B), lower back (C), and inner thigh (D). E, Averages of cerebrospinal fluid (CSF) RT-QUIC ThT fluorescence kinetics of PRPSC over time. F-J, Comparison of RT-QUIC parameters across different skin sites at various dilutions. The Tlag (F), Fmax (G), T50 (H), Vmax (I), and RT-QUIC score (J) of different skin sites at different dilutions were compared. K-O, Comparison of RT-QUIC parameters between different skin sites (at a dilution of 10^{-2}) and CSF. The Tlag (K), Fmax (L), T50 (M), Vmax (N), and RT-QUIC score (O) of different skin sites and CSF were compared.

[a] $P < .01$.

[b] $P < .05$.

[c] $P < .001$.

Table 2. Demographics, Clinical Features, and Test Results in Real-Time Quaking-Induced Conversion (RT-QUIC) in Patients With Prion Diseases (PRDs) With Skin+/Cerebrospinal Fluid (CSF)− and Skin+/CSF+ Status

Characteristic	Skin+/CSF− (n = 19)	Skin+/CSF+ (n = 70)	*P* value
Patient information			
Age at onset, mean (SD), y	58.1 (9.3)	61.9 (91.1)	.12
Sex, No. (%)			
Female	12 (63.2)	41 (58.6)	.72
Male	7 (36.8)	29 (41.4)	
Onset to RT-QUIC, median (IQR), d	224.0 (51.0-289.0)	73.0 (50.8-159.5)	.06
Duration, median (IQR), mo	19.0 (14.0-21.5)	8.0 (2.8-16.0)	.02
Degree of disease progression, mean (SD)	0.47 (0.26)	0.59 (0.29)	.28
Clinical features at presentation, No. (%)			
Cognitive	17 (89.4)	68 (97.1)	.15
Psychiatric	10 (52.6)	39 (55.7)	.81
Visual	7 (36.8)	25 (35.7)	.93
Extrapyramidal	9 (47.4)	44 (62.9)	.22
Pyramidal	8 (42.1)	36 (51.4)	.47
Cerebellar	12 (63.2)	44 (62.9)	.98
Myoclonus	3 (15.8)	28 (40.0)	.049
Mutism	1 (5.3)	1 (1.4)	.38
Scale evaluation			
MMSE, median (IQR)	14.0 (0-21.0)	0.5 (0-18.8)	.11
MoCA, median (IQR)	7.5 (0-15.0)	0 (0-8.8)	.03
MRC Prion Disease Rating Scale, mean (SD)	14.2 (5.6)	10.3 (6.5)	.02
CSF studies			
RBC, median (IQR), 10^6/L	0	0	.32
WBC, median (IQR), 10^6/L	1 (1-2.3)	1.0 (1-2)	.78
Protein, median (IQR), mg/dL	29.9 (26.5-51.4)	31.8 (23.7-43.6)	.95
Glucose, median (IQR), mg/dL	64.4 (61.1-70.4)	70.1 (63.9-80.0)	.047
CSF tau >1150 pg/mL, No. (%)	9/14 (64.3)	43/51 (84.3)	.10
14-3-3 positive, No. (%)	15 (78.9)	49 (70.0)	.44
Periodic discharges on EEG, No. (%)	3 (15.8)	37 (52.9)	.009
Hyperintensity on DWI, No. (%)	14 (73.7)	69 (98.6)	<.001
Cerebral cortex	14 (73.7)	68 (97.1)	.001
Basal ganglia	1 (5.3)	31 (44.3)	.002
Thalamus	0	7 (10.0)	.15
Codon 129 MM genotype, No. (%)	19 (100.0)	68 (97.1)	>.99
Genetic prion diseases	3 (15.8)	9 (12.8)	.74
Probable MM2 type SCJD	8/16 (50.0)	11/61 (18.0)	.008

Abbreviations: CSF, cerebrospinal fluid; DWI, diffusion-weighted imaging; EEG, electroencephalography; MMSE, Mini-Mental State Examination; MM2, methionine homozygous at codon 129 with PRPSC type 2; MoCA, Montreal Cognitive Assessment; MRC, Medical Research Council; RBC, red blood cell; SCJD, sporadic Creutzfeldt-Jakob disease; WBC, white blood cell.

Mismatch of PRPSC-SA Between CSF and Skin

There were 20 of 94 cases (21.3%) with discordant results between CSF and skin RT-QUIC in the exploratory cohort. The proportion of CSF-negative and skin-positive RT-QUIC assay was as high as 20.2% (19 of 94), whereas the proportion of CSF-positive and skin-negative cases was 1.1% (1 of 94) (eTable 5 in Supplement 1).

The comparison of clinical features and PRPSC-SA between patients with RT-QUIC skin+/CSF− and skin+/CSF+ status is detailed in **Table 2**. Among the 19 patients with negative CSF RT-QUIC results, 16 were diagnosed with SCJD, and 3 patients were diagnosed with genetic PRDs. Patients with skin+/CSF+ status exhibited a significantly shorter disease duration compared with patients with skin+/CSF− status (8.0 [IQR, 2.8-16.0] vs 19.0 [IQR, 14.0-21.5] months; *P* = .02) and more severe clinical symptoms, including lower Montreal Cognitive Assessment scores (0 [IQR, 0-8.8] vs 7.5 [IQR, 0-15.0]; *P* =.03), lower Medical Research Council Prion Disease Rating Scale scores (mean [SD], 10.3 [6.5] vs 14.2 [5.6]; *P* =.02), and a higher incidence of myoclonus 28 of 70 [40.0%] vs 3 of 19 [15.8%]; *P* =.049). Specific CJD tests, such as periodic discharges on electroencephalography and hyperintensity on diffusion-weighted imaging, were more common in the group with skin+/CSF+ status (52.9% [37 of 70] vs 15.8% [3 of 19]; *P* =.009; 98.6% [69 of 70] vs 73.7% [14 of 19]; *P* <.001). Additionally, the proportion of clinically diagnosed probable MM2-type SCJD was significantly lower in the group with skin+/CSF+ status compared with those with skin+/CSF− status (18.0% [11 of 61] vs 50.0% [8 of 16]; *P* = .008). Patients with skin+/CSF+ status had shorter Tlag and higher T50 values and higher RT-QUIC thioflavin T intensity compared with patients with skin+/CSF− status, whereas Fmax and Vmax showed

no significant differences between the 2 groups (eFigure 5 in Supplement 1).

In this study, there were 5 patients with false-negative skin RT-QUIC assay, one of whom had a positive CSF RT-QUIC assay. The clinical and auxiliary examination conditions of each patient on admission are shown in eTable 6 in Supplement 1. Patients 1, 3, and 4 did not meet the criteria for possible SCJD on admission due to insufficient clinical symptoms but met the criteria for probable SCJD during follow-up as their symptoms progressively worsened.

Discussion

Results of this diagnostic study, which was, to our knowledge, the largest to date comparing skin biopsy samples with CSF prion SA for diagnosing PRDs, suggest that individual skin RT-QUIC analysis was as effective as CSF RT-QUIC at a single dilution. Using multigradient dilutions of skin samples improved diagnostic accuracy. Combination of 2 or more skin samples using RT-QUIC surpassed CSF analysis alone in diagnosing PRDs. Although Tlag, T50, and RT-QUIC scores of certain skin sites correlated with cognitive impairment, neither skin nor CSF RT-QUIC parameters predicted disease prognosis.

Skin samples have recently been used to detect misfolded proteins in neurodegenerative diseases, demonstrating high sensitivity and specificity.[4,22,23] Previous studies have indicated that RT-QUIC test results vary across different skin sites, with higher positivity rates at proximal locations.[9] Our findings also confirm that skin samples from the area near the ear have the highest positivity rate and the greatest consistency in results. Our study also emphasizes that sampling from 2 or more skin sites or a single skin site with the CSF significantly improved the diagnostic efficacy for PRDs. Although the CSF has been widely used for RT-QUIC testing with high clinical value, it has been reported to have approximately up to 10% false-negative results[9] even with second-generation RT-QUIC, and some patients may not be able to have a lumbar puncture. Our study results suggest that sampling from 2 or more skin sites may improve diagnostic accuracy. Given these findings, skin biopsies show great potential as a valuable complement to RT-QUIC testing, especially when CSF testing is negative or not feasible. The skin-based method not only may enhance early detection but also provides a less invasive alternative for patients who are unable to undergo lumbar puncture, thereby broadening the clinical application of diagnosing PRDs.

Previous studies on skin RT-QUIC mainly used a single dilution concentration for testing.[5,9] The positivity rate of RT-QUIC is closely related to PRPSC content, but excessively high concentrations may inhibit the reaction. Like PRPSC-SA in brain tissue, higher concentrations do not always yield higher positivity rates; typically, dilutions up to 10^{-6} to 10^{-8} are most effective.[9,24] In our study, when skin samples were negative at a dilution of 10^{-2}, 5.4% to 12.0% of the samples showed positivity at dilutions of 10^{-3} and 10^{-4}. Similar observations were seen in CSF RT-QUIC testing, where some patients with abundant PRPSC-SA showed limited or no reactivity.[24] Therefore, for patients with negative results at a single dilution, retesting with serial dilutions may improve the detection efficacy.

CSF prion seed-amplification assay sensitivity varies from 73%[25] to 89%[6,26,27] using first-generation RT-QUIC, whereas second-generation RT-QUIC has shown sensitivity rates improved up to 92% to 97%.[2,28] Recent studies[9,29,30] on European and American populations have reported sensitivities of CSF RT-QUIC ranging from 88.5% to 90%. However, the sensitivity of CSF PRPSC-SA was found at about only 75.5% or less in our present study. The reasons for the lower CSF RT-QUIC positivity rate in China remain unclear. Notably, a high positivity rate of 96.7% was observed in CSF samples from CJD and non-CJD cases diagnosed neuropathologically, provided by the National Prion Disease Pathology Surveillance Center, Case Western Reserve University, Cleveland, Ohio, under identical RT-QUIC experimental conditions, effectively ruling out technique issues as a contributing factor.[31] We hypothesize that this discrepancy may be related to the prevalence of specific SCJD subtypes in China, where the prevalence of the MM2-type SCJD may approach one-fourth, as observed in our previous study.[32] Previous studies have indicated false-negative rates of CSF RT-QUIC in MM2-type SCJD ranging from 21% to 25%.[9,29,30] However, this does not explain the low positivity rate in patients with genetic PRDs. We speculate that differences in CSF components among ethnic groups might suppress RT-QUIC reactions. For example, elevated apolipoprotein levels have been found to affect α-synuclein RT-QUIC reactions.[33] Although it is unclear if apolipoproteins affect prion seeding, previous research has confirmed significant differences in blood apolipoprotein levels between ethnic groups.[34-36]

Patients with negative CSF RT-QUIC results in our study tended to have longer disease durations than those with positive results, aligning with a higher proportion of MM2-type SCJD in this subset, known for longer survival.[37] We observed a lower proportion of myoclonus among patients with false-negative CSF results, whereas previous studies[28,30] have indicated that these patients are typically less often associated with ataxia and motor disturbances.[30] Electroencephalography triphasic waves and high magnetic resonance imaging signal intensity were also less common in patients with negative CSF RT-QUIC, likely due to including FFI- and MM2T-type cases, where these tests are rarely positive. Although elevated CSF protein[24] and red blood cells[2] are linked to CSF RT-QUIC false-negative results, we found no differences in protein levels in CSF samples of our patients.

RT-QUIC parameters can differentiate subtypes of synucleinopathies,[38,39] but their correlation with clinical parameters and pathological changes varies.[19,40,41] Similar to synucleinopathies, different PRD subtypes exhibit significant differences in their RT-QUIC amplification curves.[2] Our preliminary findings suggest that skin RT-QUIC parameters may partially reflect cognitive impairment in PRDs. However, RT-QUIC parameters in both skin and CSF were found to be unrelated to survival time and disease progression, indicating limited utility in prognostic assessment and disease monitoring. Patients who tested positive for RT-QUIC showed a shorter

survival,[28,30] although this may be related to the pathological subtype.

Limitations

There are several limitations to consider in this study. First, all patients with SCJD were clinically diagnosed without pathological confirmation, although regular follow-up was conducted to ensure diagnostic accuracy. Second, SCJD was categorized based on clinical diagnostic criteria, which may differ from neuropathological findings. Moreover, a significant proportion of SCJD cases exhibit mixed PRPSC types, which could impact the results.[37,42,43] Third, we initially randomly sampled 3 of 4 sites for testing and found the lowest positivity rate in the upper arm. Subsequently, we modified the sampling sites, resulting in a design flaw and uneven sample sizes from different skin sampling points. Lastly, we did not dilute CSF in our study, but based on our experience with skin sampling, it is most likely that CSF dilution may also help increase the positivity rate.

Conclusions

The findings of this diagnostic study suggest that analysis of 2 or more skin sites was superior to CSF analysis in diagnosing PRDs. Although skin testing cannot entirely replace CSF RT-QUIC in PRDs, skin RT-QUIC is particularly useful for patients with negative CSF RT-QUIC results or those with contraindications for or refusal of lumbar puncture. RT-QUIC parameters may be associated with cognitive impairment in PRDs. Whether they can also serve as prognostic or disease monitoring indicators remains to be further investigated with more cases.

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